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T e r e s a P O J M A Ń S K A

Metacercariae of some *Brachylaemidae* (Trematoda) in land snails of the Białowieża National Park

Metacerkarie niektórych *Brachylaemidae* (Trematoda) u ślimaków
lądowych Białowieskiego Parku Narodowego

As a continuation of studies by Sołtys (1951, 1953), Żarnowski (1955) and ourselves (Pojmańska, 1956, 1957) on the parasites of small forest mammalians we started in the spring of 1958 the collection of data on life cycles of flukes parasitic in these hosts*. Taking account of the biotope of the final hosts we took up research on land snails as the most likely intermediate hosts of flukes of small mammals.

M a t e r i a l

During the spring, summer and autumn we examined 2287 snails of 18 species of which only 4 have so far proved intermediate hosts of flukes (Table I).

The snails were collected in the Białowieża National Park in two rather different biotopes:

- a mixed forest with a fairly moist foundation. Most snail species came from that site and they were found under the bark of felled trees and fallen leaves;
- a meadow on the river Orlówka running through the forest. The meadow was overgrown with reeds, thick grass and nettles, and scanty shrubs. The snails were collected from plants, grass and slime from the river bank. All specimens of *Succinea putris*, *Perforatella bidens*, *Eulota fruticum*, *Zenobiella rubiginosa*, *Galba trun-*

* We offer thanks to Prof. Dr A. Dehnel for admitting us to a studio at the Białowieża Institute for Mammals and helping us with arranging for field work which unabled us to carry out this research.

Table I
Extensivity of invasion of examined snails

Hosts	No. of specimens examined	No. of specimens invaded	% of invasion	Metacercaria
<i>Goniodiscus rotundatus</i> (Müll.)	161	1	—	<i>B. fulvus</i> Duj.
<i>Goniodiscus ruderatus</i> (Stud.)	153	1	—	<i>Leucochloridium</i> sp.
<i>Zonitoides nitidus</i> (Müll.)	539	26	4.1	<i>B. fulvus</i> Duj.
<i>Perpolita petronella</i> (Pfr.)	3	—	—	—
<i>Vitrea cristallina</i> (Müll.)	13	—	—	—
<i>Vitrea contracta</i> (West.)	2	—	—	—
<i>Vitrina pellucida</i> (Müll.)	13	—	—	—
<i>Eulota fruticum</i> (Müll.)	154	—	—	—
<i>Perforatella bidens</i> (Chemn.)	491	6	1.22	<i>P. soricis</i> (Soltys)
		2	0.41	<i>B. spinosulus</i> H.
<i>Zenobiella rubiginosa</i> (Schm.)	1	—	—	—
<i>Succinea putris</i> (L.)	329	6	1.81	<i>P. soricis</i> (Soltys)
		14	4.24	<i>B. spinosulus</i> H.
		2	0.64	<i>L. paradoxum</i> (Carus)
<i>Carychium minimum</i> (Müll.)	8	—	—	—
<i>Euconulus trochiformis</i> (Mont.)	20	—	—	—
<i>Cochlicopa lubrica</i> (Müll.)	19	—	—	—
<i>Galba truncatula</i> (Müll.)	2	—	—	—
<i>Clausilidae</i> sp.	346	—	—	—
<i>Limacidae</i> sp.	10	—	—	—
<i>Arionidae</i> sp.	13	—	—	—

catula, and a few specimens of *Zonitoides nitidus* and *Clausilidae* came from this site.

The intensity and extensivity of invasion was low. Most of the snails collected were not invaded. But since the extensivity of invasion was low it was necessary to collect a great number of snails (not just a few scores as is sometimes the case) to find out whether they were intermediate hosts of flukes.

Only metacercariae (in various stages of development) were found in the snails invaded. But we found no sporocysts or cercariae (with

the exception of two specimens of *Succinea putris* invaded by sporocysts of *Leucochloridium paradoxum*, which we shall not discuss here in detail). The metacercariae belonged to four fluke species, all of the family *Brachylaemidae* Stiles et Hassall, 1898.

One and the same fluke species most often occurred in one and the same snail species. Yet *Brachylaemus fulvus*, which occurred mainly in *Zonitoides nitidus*, was found once also in *Goniodiscus ruderatus* from the same place and *Brachylaemus* sp. and *Pseudoleucochloridium soricis*, which occurred mainly in *Succinea putris*, were also found in *Perforatella bidens*. These facts are incongruous with a great specificity of fluke larvae claimed by Sinitzin (1931), who was of the opinion that parthenite might parasite in one snail species only. Adam and Leloup (1934) were the first to disprove this statement as regards *Brachylaemus helcis-pomatiae* (Diesing, 1850).

One species of metacercariae usually occurred in one and the same specimen of snail, yet in one case *Brachylaemus* sp. occurred together with *Pseudoleucochloridium soricis*.

Succinea putris proved to be the most „non-specific” snail species because it was an intermediate host of three different fluke species.

Methods

With their shells removed the snails were crashed in a drop of physiological salt between two slides under a binocular or microscope. If a snail was not crushed too much the localization of metacercariae could be determined. When detected every metacercaria was passed on to another slide in a thin pipette and then observed and measured while alive. In order better to observe their internal organs live flukes were stained with neutral red in a 1: 100,000 concentration. The armature, if any, of the cuticle, the position of the genital pore and the excretory system could be well observed in live material. These particulars are difficult to determine in preserved material. Then every metacercaria was killed when the water was warmed up. This method, used by McMullen (1937), Denton (1944) and Miller (1954) has advantage of killing flukes with muscles relaxed so that they were neither very contracted nor extended. The larvae were stained in alum carmine and closed in balsam for further observation.

Detailed data

Metacercaria of *Brachylaemus fulvus* Duj., 1843

This metacercaria was fairly common in *Zonitoides nitidus* (Müll.): in 26 out of 531 specimens examined. Moreover it was found in one out of 165 specimens of *Goniodiscus rotundatus* (Müll.) examined. The intensity of invasion was very low; usually one, sometimes two, once six metacercariae were found in a snail.

Description. A fairly large, whitish, very mobile metacercaria with a very thick cuticle. Fore part of the body always rounded, the hind part rounded, or very narrow, depending on the contraction of the body. Dead specimens, if not very much contracted, are always widest at the level of the ventral sucker. Cuticle covered with fine spines up to the level of the centre of the ventral sucker. These spines are arranged in regular rows, denser in front and over scarcer further on. From the level of the ventral sucker as far as the body end the cuticle forms transverse folds, more or less distinct, depending on the contraction of the body. Both suckers are of almost the same size, the oral always somewhat smaller than the ventral, which lies in the fore third of the body. Pharynx large, well-muscled. Oesophagus invisible at normal contraction, very short at considerable extension. Intestines thin not reaching the hind body end. Reproductive organs well developed. Testes and ovary spherical, more or less of the same size, lying one behind the other or forming a triangle depending on the contraction of the

Table II

Dimensions of *Brachylaemus fulvus* Duj. in mm

Measurements	one (and the same) specimen		variation range of dimensions of organs
	live	preserved	
length	1.3	1.2	1.0 —1.45
width	0.44	0.42	0.40—0.48
oral sucker	0.182 × 0.208	0.14 × 0.14	0.10 —0.184 × 0.14 —0.19
ventral sucker	0.182 × 0.221	0.16 × 0.16	0.16 —0.22 × 0.16 —0.222
pharynx	0.065 × 0.065	0.08 × 0.08	0.045—0.08 × 0.045—0.08
testis I	*	0.048 × 0.044	0.044—0.052 × 0.044—0.056
testis II	*	0.046 × 0.046	0.033—0.073 × 0.033—0.08
ovary	*	0.05 × 0.044	0.024—0.052 × 0.033—0.052

* The size of the testes and ovary in live specimens were not measured.

body, between the intestines in the last quarter of the body. Cirrus sac outlined. Genital pore usually very distinct, lying on the medial line or somewhat shifted aside, usually in front of the anterior testis, sometimes on the level of its anterior border. Uterus run-

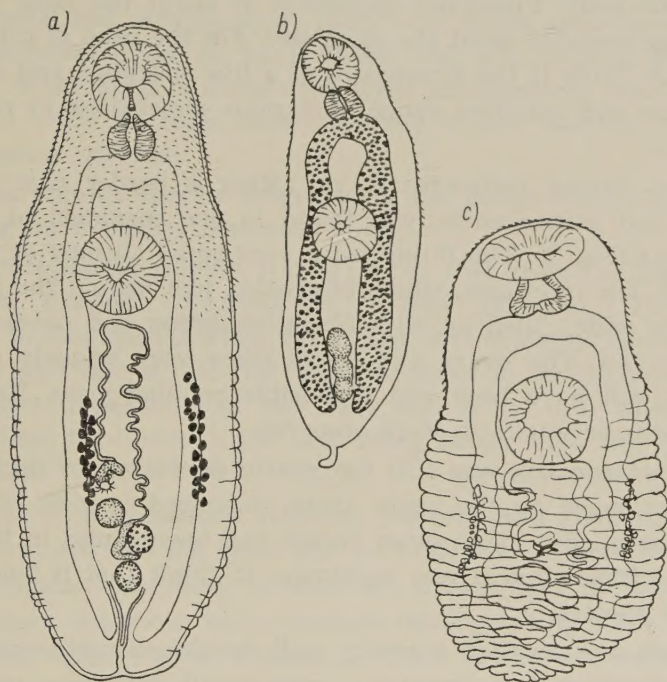


Fig. 1 a — Metacercaria of *Brachylaemus fulvus* Duj., 1843; b — Very young specimen with rudimentary tail and intestine filled with food; c — Contracted specimen, foldings of the cuticle.

ing in mild bends to the ventral sucker, turning back at its posterior border and descending to the genital pore, without reaching in front of the ventral sucker. Vitellaria lying laterally, usually on the intestines, forming very short bands reaching forwards about half-way between the genital pore and the ventral sucker and at the back to the level of the middle of the anterior testis or the ovary. Vitelline bands frequently not quite symmetrical (Fig. 1).

The chief canals of the excretory system (observed in vivo) run intracaecally. Excretory bladder short, tubular, bifurcating at the level of the ends of the intestine.

Well-developed metacercariae prevailed in the material collected but younger ones were also found with uterus less distinct and without vitellaria.

Live specimens varied widely in size depending on the contraction of the body. Preserved specimens in about the same development stage were of about the same size. For the sake of comparison we give in Table II the dimensions of a live specimen and of a preserved one and variation range of various dimensions in preserved specimens.

A very young metacercaria, or rather a border case between cercaria and metacercaria, was found in one specimen of *Zonitoides nitidus* (Fig. 2). The fluke had a very small stumpy at the hind body end. The intestines, which bifurcated just behind the pharynx, were very wide, filled up with fine granulation and never reached the body end. The ovary and testes were very vaguely outlined. It might still have been one indivisible cellular mass. No genital pore, uterus or vitellaria were observed.

The metacercaria was still too poorly developed to decide whether it belonged to the same above described species of mature metacercariae. But as no other flukes had been found in that snail species at Białowieża it was legitimate to think that it belonged to the same species.

Also on one occasion a pretty well developed metacercaria was discovered which had however a very small detaching tail at the hind body end.

It may be concluded from the above described development forms that the metacercaria of this species have a rudimentary little tail which falls away as a rule on transformation into a metacercaria but sometimes may persist still in that stage. (A similar behaviour of the tail not only in a metacercaria but also in one adult fluke was referred to by Hofmann (1899) in his study on the development of *Distomum leptostomum* Olsson).

Discussion. The metacercaria described shows the following features which can be regarded as specific: the armature of the cuticle reaching the level of the centre of the ventral sucker, the relation between the dimensions of the suckers (the oral sucker always somewhat smaller than the ventral), the course of the uterus (which never reaches in front of the ventral sucker), and the

range of the vitellaria (from the anterior testis half way through the distance between the genital pore and the ventral sucker).

A tendency to form transverse folds on the cuticle we would also consider to be one of these features. These folds appeared more or less distinctly depending on the contraction of the body in live material and always, apart from very young specimens, in preserved material (Figs. 1a, b). If there were any folds in live specimens of other species discovered their arrangement was different (Fig. 2c), while no folds were observed in the material preserved by the same method.

Of the various species described of the genus *Brachylaemus* the following have similar features:

(1) *Brachylaima fulvum* Dujardin, 1843 from the intestine of *Crocidura russula* (Hermann, 1870)* (France). Dujardin referred to this species when describing *Branchylaima advena* Duj., 1843 and said that it differed from the latter in having smaller eggs with larger dimensions of the body and in its oral sucker being smaller than the ventral (the oral being larger than the ventral in *B. advena*).

Dujardin described *Distoma migrans* and *Distoma migrans* var. *a* from one and the same host in 1845. The differences between these coincide with those between *B. advena* and *B. fulvum*. The author gives one more major feature distinguishing these two species — the uterus never reaches in front of the ventral sucker in *D. migrans* var. *a*.

(2) *Harmostomum dujardini* Baer, 1928. Baer found this fluke in *Sorex araneus tetragonurus* in Switzerland and regarded it as identical with *Distoma migrans* var. *a* Duj., 1845 and *Brachylaima fulvum* Duj., 1843, but because of inaccurate description he considered the latter name to be a nomen nudum (just as *B. advena* in relation to *B. migrans* Duj., 1845**).

* We quote this host after Dollfus (1934), who maintains that „la musaraigne musette”, *Sorex araneus*, referred to by Dujardin, coincides with *Crocidura russula* Hermann, 1870.

** Dollfus (1934) regards *B. migrans* var. *a* Duj., 1845 as a synonym of *B. fulvum* Duj., 1843 as well but maintains the latter name as he thinks Dujardin's description or rather his differential diagnosis to warrant the maintenance of its priority. Adam and Leloup (1935) are of the same opinion. As regards *Harmostomum dujardini* Baer, Dollfus is not sure

The fluke described by Baer under this name closely resembles the metacercaria we describe in that it has spines in the fore section of the body and a folded cuticle in the hind section. It also resembles our metacercaria in relative and absolute dimensions of the suckers, the shape and position of the genital glands, the range of the uterus and vitellaria. It differs however in the position of the genital pore, which lies at the level of the ovary, and in wider intestines, the latter feature being rather irrelevant.

Baer also found metacercaria in *Agriolimax agrestis* (L.), which in his opinion coincided with that fluke. Without giving their drawing he states that they are 1.2 mm long, 0.3 mm wide, their suckers are almost equal, 0.19 mm in diameter.

(3) *Brachylaemus fulvus* Dujardin 1843, described by Dollfus (1934) from *Sorex araneus* (L.), from France. Dollfus's drawing closely resembles Baer's, yet the genital pore lies in front of the anterior testis, just as found by Dujardin in *B. fulvum* and as the case is with our metacercariae. The only difference consists in the range of the vitellaria, which almost reach the ventral sucker in Dollfus's drawing. Yet shifts in the front range of the vitellaria may be due to strong contraction. The vitellaria also reached almost as far as the ventral sucker in the metacercaria described (Fig. 1c).

(4) *Panopistus europaeus* Soltyś, 1951 — from the intestine and pharynx of *Sorex araneus* L., *Sorex minutus* L. and *Neomys fodiens* Schreb. from Białowieża, Poland. The fluke was wrongly assigned to the species *Panopistus Sinitzin*, 1931.

On examination of Soltyś's preparations from which he described the fluke we could see that its structure fully coincides with our metacercaria. The position of the genital pore in front of the anterior testis suggests that it is a fluke of the genus *Brachylaemus* *.

whether it can be treated as synonyms of *B. fulvum* considering the position of the genital pore (between the testes). Should it prove that it was an individual not a specific feature, the name should, according to Dollfus, be assigned to synonyms of *B. fulvum*. Adam and Leloup regard the name as a synonym of *B. fulvum* and the present author is inclined to accept the last view.

* I would like to thank warmly Dr A. Soltyś for making his preparations available to me, thanks to which I could state the identity of our metacercaria and flukes from Białowieża.

The size, armature, shape of the body, range of the uterus and vitelline glands suggest that these specimens belong to the species *Brachylaemus fulvus* Duj., 1843.

(5) *Brachylaemus oesophagei* Shaldibin, 1953 — from the stomach, oesophagus and intestine of *Sorex araneus*, *S. minutus* and *Neomys fodiens* collected in the European part of the USSR. This fluke, just as the one described above, has spines reaching to the level of the ventral sucker and farther on the cuticle forms foldings; the loops of the uterus reach only as far as the level of the centre of the ventral sucker. Only the border of the vitelline glands reaches somewhat farther than in the forms discussed above as it reaches to the level of the anterior border of the posterior testis. The dimensions of the various organs and the size of the eggs coincide with the dimensions quoted by Baer (1928).

On the basis of the survey made we think that the following should be regarded as synonyms of *Brachylaemus fulvus* Duj., 1843: *Brachylaemus migrans* var. *a* Duj., 1845, *Panopistus europaeus* Soltyś, 1951; *Brachylaemus oesophagei* Shaldibin, 1953, and probably *Harmostomum dujardini* Baer, 1928*.

The final hosts of this fluke are the following: *Crocidura russula* Hermann, 1780, *Sorex araneus* L., *S. minutus* L. and *Neomys fodiens* Schreb.

The land snail *Zonitoides nitidus* (Müll.) is its intermediate host in the area of the Białowieża Forest.

Metacercaria of *Brachylaemus spinosulus* Hofmann, 1899

We found this metacercaria in two snails: *Succinea putris* (L.) and *Perforatella bidens* (Chemn.) collected at the same site at the river Orłówka. *Succinea* was more heavily invaded (14 specimens out of 329 examined), *Perforatella* less heavily (2 specimens out of 491 examined). The intensity of invasion in *Succinea putris* was on the whole higher than the intensity of invasion by *B. fulvus* in *Zonitoides nitidus*. Usually there were 4—5 specimens in one snail, but sometimes also 10, 11, and 21. There was only one metacercaria in *Perforatella bidens* in both cases.

Description. A fairly large, slender, whitish, very mobile metacercaria. Anterior part of the body rounded and, as rule, wider

* I make this reservation because of the situation of the genital pore in Baer's description.

than the posterior. Cuticle in the anterior part covered with small spines arranged in regular rows. In the ventral side they are thicker and occupy the area of the ventral sucker (Fig. 2a); on the dor-

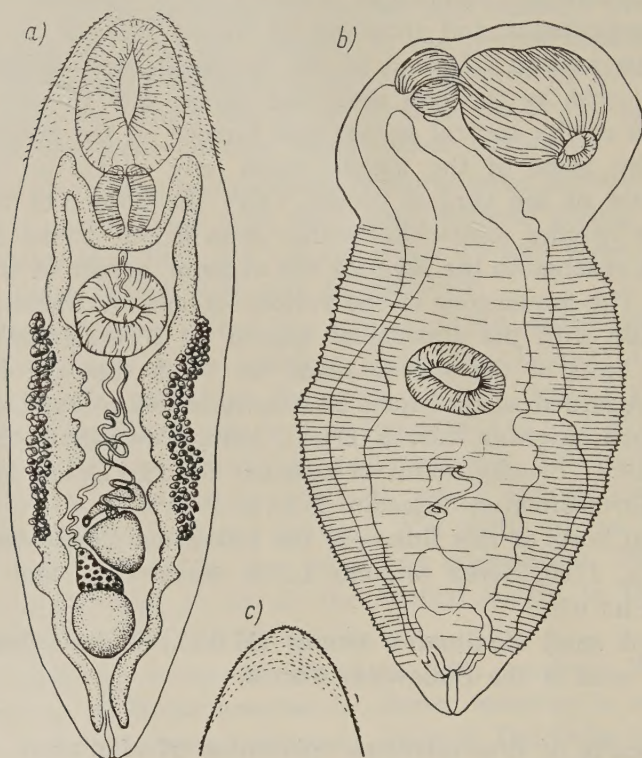


Fig. 2. Metacercaria of *Brachylaemus spinosulus* Hofmann, 1899; b — Armature of the dorsal side of the body; c — Contracted specimen, foldings on the cuticle.

sal side they are thinner and limited to a small part only (Fig. 2b). In live specimens, when the body contracts, the cuticle of the metacercariae forms transverse foldings, which are however quite different those in *Brachylaemus fulvus* metacercariae. They are convex, with sharply protruding edges, so that the lateral edges of such fluke are deeply indented (Fig. 2c). These foldings never occur in the preserved material. Numerous, strongly staining globules are distinct in the whole body and are also distinct in live specimens. (Hofmann, 1899, mentioned similar formations when he described *Cercariaeum helcis* and *C. spinosulum* and so did Krull,

Table III
Dimensions of *Brachylaemus spinosulus* metacercariae

Measurements	live specimens	preserved specimens
length	1.20 — 1.90	0.81 — 1.44
width	0.46 — 0.70	—0.24 — 0.56
oral sucker	0.28 — 0.3 × 0.3 — 0.4	0.15 — 0.28 × 0.19 — 0.32
ventral sucker	0.20 — 0.24 × 0.20	0.104 — 0.196 × 0.113 — 0.196
pharynx	0.12 — 0.14 × 0.20 — 0.24	0.072 — 0.139 × 0.081 — 0.164
testis I	—	0.038 — 0.128 × 0.051 — 0.134
testis II	—	0.043 — 0.126 × 0.049 — 0.128
ovary	—	0.031 — 0.109 × 0.051 — 0.123

1934, when discussing *Brachylaemus virginiana*). The oral sucker is always much larger than the ventral (see Table III). The ventral sucker is situated in the anterior part of the body. The pharynx is only slightly smaller than the ventral sucker. The prepharynx and the very small oesophagus could be observed in live and stretched specimens. In the preserved material the intestine bifurcates just behind the pharynx. The intestines are wider than in the species discussed before, they reach almost as far as the end of the body and form numerous large foldings. Numerous very distinct glandular cells appear near the bifurcation of the intestine. Reproductive organs well developed on the whole. Large testes of irregular shapes (from almost triangular to spherical) lie in the third posterior part of the body. The ovary lies between them and is always smaller than the testes. The cirrus sac lies in front of the anterior testis. The genital pores, lying closely to each other, vary somewhat in position: from the level of the middle of the anterior testis to the position rather far in front of the anterior testis, at the central line, or somewhat shifted. The uterus reaches as far as the bifurcation of the intestine, forms numerous loops and goes down to the female genital pore. Vitellaria well developed. Their range varies in various specimens. The anterior end of the vitellaria usually reaches as far as the level of the centre of the ventral sucker, sometimes however as far its anterior border or even the level of the pharynx. The posterior end usually lies at the level of the anterior border of the anterior testis, sometimes reaches as far as the middle of the anterior testis, or as far as the level of the anterior border of the ovary.

The excretory bladder is rather short, tubular or mace-like. The main excretory ducts always lie intracaecally.

In *Succinea putris* of the same site we found younger forms without well-developed vitellaria and a hardly outlined uterus but quite similar to the cercariae described and very young forms transitory between cercaria and metacercaria (Fig. 3b).

Those forms were considerably smaller and had a small stumpy tail. They were covered with very fine spines which were particularly distinct in front and on the tail. The oral sucker was somewhat larger than the ventral. The intestines, thick and filled up with fine granulation, reached almost as far as the body end. We found these young forms in four specimens of *Succinea putris*, a few in each of the specimens. In two cases they occurred together with mature metacercariae.

Also Krull (1935a) refers to young immature metacercariae found together with mature ones when he describes the development of *Brachylaemus virginiana*, and so do Adam and Leloup (1934) when describing the metacercariae of *Brachylaima recurvum* (= *B. spinosulum* Hofmann). Both of these authors state that those had a small tail covered with fine spines*.

Among the adult metacercariae we also found one with a rudimentary tail which fell off during preservation. Unfortunately we failed to observe in vivo whether it had been covered with spines or not.

Finally we found a cystless metacercaria (Fig. 3b) in one *Succinea putris* collected in September 1957. It was considerably shrunk but not coiled in its cyst. Its suckers were very close to each other, the pharynx partly covered the oral sucker, the intestines formed strong folds, the vitellaria reached from the middle of the anterior testis as far as the posterior border of the pharynx. Dimensions: length 0.92 mm, width 0.526 mm, oral sucker 0.274×0.232 mm, ventral sucker 0.125×0.203 mm, pharynx $0.147 \times$

* In connection with the discovery of these tailed metacercariae Dollfus (1935b) claims that Adam and Leloup did not actually encounter larval stages of *B. spinosulum*, since Hofmann, when he described the metacercariae of this species, stated that he had found no forms with a tail or any trace of it.

We think this is no sound objection. The tail is a characteristic feature of cercariae, not of metacercariae. When Hofmann described his cercariae he did not say whether he considered them cercariae of *D. leptostomum* or of *Cercariaeum spinosulum*.

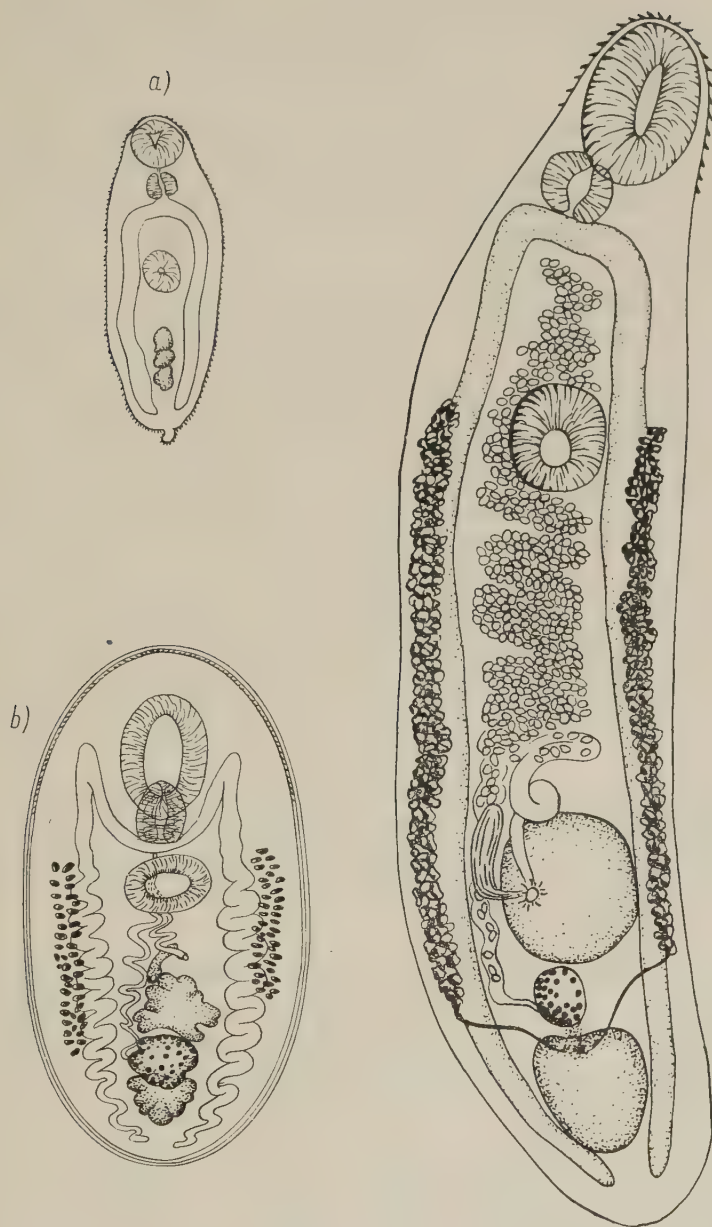


Fig. 3. *Brachylaemus spinosulus* Hofmann, 1899; a — Very young metacercaria with rudimentary tail; b — Encysted metacercaria; c — Adult specimen from hedgehog.

$\times 0.132$ mm. The phenomenon of a metacercaria getting encysted in kidney of the II intermediate host and preserving its body shape was described by Krull (1935a) with respect to *Brachylaemus virginiana*.

That metacercaria was also interesting because its ovary and testes were lobular, the anterior testis more, and the posterior and the ovary less so. The metacercaria appeared closely to resemble *Brachylaemus* sp. metacercaria described by Dollfus from *Heliella ericetorum* in 1934.

We succeeded in invading the European hedgehog, *Erinaceus europaeus*, with these metacercariae in summer 1958. Out of 15 metacercariae given we found only one fluke at a post-mortem made ten days after the invasion. That fluke was not quite mature yet. Although there were already fairly numerous eggs in its uterus, their measurements were smaller than those of mature eggs, no eggs were ever discovered in the faeces which were examined several times before the post-mortem.

The fluke had major specific features: the armature of the body appearing only in the region of the oral sucker, the oral sucker far larger than the ventral, the range of the vitellaria, from the level of the middle of the anterior testis to the level of the middle of the ventral sucker, quite similar to the same features in metacercariae.

Discussion. The metacercaria discussed and the fluke obtained experimentally show much resemblance to two fluke species: *Brachylaemus spinosulus* Hofmann, 1899 from the hedgehog intestine and *Brachylaemus virginiana* (Dickerson) Krull, 1934 from the opossum intestine.

(1) *Brachylaemus spinosulus* — The taxonomic status of this species has not been clearly established yet. Hofmann distinguished it on the basis of the appearance of spines in the front of the body from the quite similar species *B. helici-pomatiae* (Diesing, 1850) (= *Distomum leptostomum* Olsson). Baer (1928) claimed that there were no good reasons to separate these two species and described its armed form under the name of *Harmostomum* (*Harmostomum*) *helicis* (Meckel, 1846) and regarded *Cercariaeum helici* Meckel, 1846, *Distomum caudatum* Lin-stow, 1873, *Distomum leptostomum* Olsson, 1876, *Cercariaeum spinosulum* Hofmann, 1899 as its synonyms. Sinitzin (1931)

thought this species to be a synonym of *Brachylaimus migrans* Duj., 1845; he also assigned *Harmostomum opisthotrias* var. *virginiana* Dickerson, 1930 and *Brachylaimus recurvus* Duj., 1845 to synonyms of this species. Adam and Leloup (1935) regarded this species as a synonym to *B. recurvus* and also assigned *Harmostomum helici* sensu Baer and *Heterolope aequans* Looss, 1899 to its synonyms. Dollfus (1934) treats the armed form as a variety of the unarmed form and applies the name *Brachylaemus erinacei* Blanchard, 1847 var. *spinosulus* (Hofmann, 1899).

The metacercaria we found is very similar to those forms of *Brychylaemus spinosulus* described by various authors under various names. Yet certain differences may also be discovered, above all in the armature of the cuticle which in the forms described by various authors reaches behind the region of the oral sucker as far as the bifurcation of the intestine (Hofmann, 1899) or even the ventral sucker (Baer, 1928) (some of the authors give no exact extent of the armature and limit themselves to stating that it appears in the fore part of the body). The relative measurements of the suckers differ, too. On the whole the ventral sucker in descriptions and drawings by various authors is either the same as (Adam and Leloup, 1935), or only somewhat larger than the ventral (Hofmann, 1899).

(2) *Brachylaemus virginiana* (Dickerson) Krull, 1934. *Brachylaemus virginiana* was distinguished by Krull as a separate species from *B. opisthotrias* var. *virginiana* Dickerson, 1930 on the basis of such features as: smaller size, presence of spines on cuticle, ventral sucker smaller than oral, larger eggs, smaller measurements of all organs.

This species resembles the metacercaria discussed more closely. The armature of the cuticle reaches here only the level of the pharynx (Krull, 1935b), while the oral sucker is far larger than the ventral (Sinitzin, 1931, Reynolds, 1938a, Ulmer, 1952). Metacercaria in Ulmer's Fig. 4 closely resembles the cystless metacercaria we found.

In the light of the examples quoted it appears that the metacercariae we found at Białowieża resemble *Brachylaemus virginiana* more closely than *B. spinosulus*. However, taking account of the host (hedgehog for *B. spinosulus* and opossum for *B. virginiana*) and the geographical range (Europe for *B. spinosulus* and North

America for *B. virginiana*) we decided to describe this species as *Brachylaemus spinosulus*. Both of the species discussed approximate each other so closely that the question should be considered whether they are not a single species. Although Krull (1935b), when analysing flukes at one age obtained from an experimental invasion finds out that *Brachylaemus spinosulus* differs from *B. virginiana* in larger measurements or a larger ovary and in the range of the vitellaria (the posterior extremity reaching farther than the anterior border of the anterior testis), these features are practically of no major importance for the classification of material since in the case of natural invasion we do not know what the age of the fluke is, the relation between the size of the testes and that of the ovary varies with the growth of the fluke, while the range of the vitellaria, as shown by our observation, varies greatly. Features such as the range of the armature of the cuticle and the relative measurements of the suckers, which were fairly constant in our material, brought the metacercariae we found closer to *B. virgi-*

Table IV
Comparison of *B. spinosulus* and *B. virginiana*

Measurements	<i>Brachylaemus spinosulus</i>			<i>B. virginiana</i> Krull
	Hofmann*	Krull	Pojmańska	
length	2 — 2.5	2.75	1.83	2.60
width	0.5	0.482	0.448	0.315
oral sucker	0.06	0.261×0.225	0.254×0.213	0.227×0.208
ventral sucker	0.04	0.214×0.237	0.184×0.184	0.178×0.203
relation between oral and ventral suckers		1.1 : 1	1.3 : 1	1.42 : 1
pharynx		0.131	0.116×0.114	0.106
testes	0.3	0.326×0.27	0.190×0.118	0.235×0.181
ovary	0.07	0.145×0.202	0.099×0.099	0.122×0.113
eggs	0.03×0.015	0.033×0.021	0.022×0.014	0.035×0.020
armature of cuticle	as far as bifurcation of intestine		as far as level of pharynx	as far as level of pharynx

* I suppose that in the measurements of the ventral and oral suckers as given by Hofmann there are misprints.

niana, to which we cannot agree considering the host and the geographical range. It seems that before it is decided whether we have here one or two species, the value of the discussed taxonomical features ought to be analysed in detail from the point of view of their individual variability in intermediate and final hosts in various biotopes and various geographical latitudes.

Table IV shows a comparison of measurements of adult *Brachylaemus spinosulus* and *B. virginiana* as made by various authors.

Succinea putris (L.) and *Perforatella bidens* (Chemn.) were the main intermediate hosts of *B. spinosulus* at Białowieża. Moreover it was once found in *Eulota fruticum* (Müll.). *Perforatella bidens* and *Eulota fruticum* are new intermediate hosts of this fluke.

Metacercaria of *Pseudoleucochloridium* gen. nov. *soricis*
(Sołtys, 1951)

This metacercaria occurred in two snail species collected on the Orlówka: *Succinea putris* (L.) and *Perforatella bidens* (Chemn.). (In 6 *Succinea* out of 329 specimens examined and in 6 *Perforatella* out of 491 specimens examined.). The intensity of invasion was very small: 1—2 metacercariae. There were five of them in one snail only and in one case one metacercaria occurred together with seven *Brachylaemus spinosulus* metacercariae.

Description. A fairly large, oval, unarmed metacercaria with a fairly thick cuticle (for measurements see Table V). Suckers very large, strongly protruding, the oral always somewhat smaller than the ventral (yet the measurements of the suckers varied fairly widely). The oral sucker has usually a round opening, the ventral a slit. The oral sucker lies subterminally, the middle of the ventral sucker somewhat behind the middle of the body. Its anterior border is situated right behind the pharynx. Pharynx relatively small, muscled, often partly covering the oral sucker. Intestine bifurcating right behind the pharynx. Intestines running horizontally or even somewhat towards the front first and then towards the back, almost parallel to the sides of the body and at a long distance from them. Always straight in the anterior part, they may form small folds in the posterior. Reproductive organs lying behind the ventral sucker. Testes and ovary varying rather considerably in size and shape; they may be as large as each other or the anterior is larger than the other glands, their shape varying from triangular to oval or

Table V
Measurements of *Pseudoleucochloridium soricis* (Softys) in mm

Measurements	live	preserved
length	1.50	0.72 — 1.6
width	0.98	0.52 — 0.96
oral sucker	0.40 × 0.38	0.21 — 0.32 × 0.22 — 0.43
ventral sucker	0.46 × 0.48	0.24 — 0.46 × 0.25 — 0.49
pharynx	0.18 × 0.12	0.07 — 0.12 × 0.096 — 0.16
testis I	—	0.059 — 0.18 × 0.08 — 0.16
testis II	—	0.064 — 0.126 × 0.064 — 0.152
ovary	—	0.051 — 0.16 × 0.068 — 0.131

spherical. In three cases the testes and ovary were even found to be lobular. An outline of the cirrus pouch usually appearing behind the posterior testis. The cirrus pouch opening on to the genital sac which opens outside through one pore lying ventrally, usually at the level of the intestinal crura or somewhat higher. Uterus running from the ovary to the left, then rising in very small coils along the

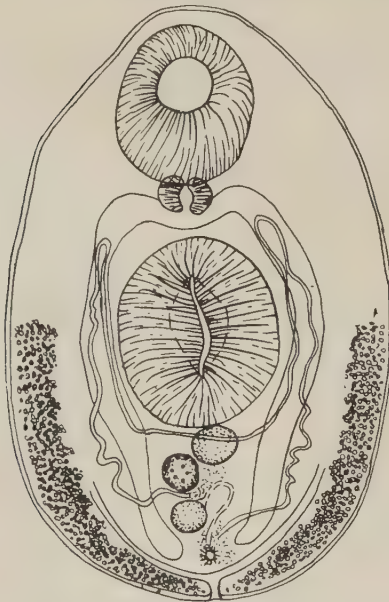


Fig. 4. Metacercaria of *Pseudoleucochloridium* nov. gen. *soricis* (Softys, 1951).

intestinal crura to the level of the anterior border of the ventral sucker to descend backwards in an abrupt swerve. At the level of the anterior testis it swerves to the right and passes horizontally on to the other side of the body, where it forms another such loop as on the left side and descends backwards to the genital pore. Vitellaria consisting of fine vesicles, lying laterally and stretching from the posterior extremity of the body as far as the level of the middle of the ventral sucker. Excretory pore lying subterminally. Excretory bladder short and tubular, main collectory canals lying extracaecally between the intestinal crura and vitellaria (Fig. 4).

Discussion. The metacercaria described is, as we could see on examination of Sołtys's preparations, the metacercaria of a fluke from the intestine of *Sorex araneus* L. coming from Białowieża and described under the name of *Leucochloridium soricis* Sołtys, 1951. We think it wrong to assign this species to the genus *Leucochloridium* Carus, 1835.

It is true that this fluke closely resembles representatives of the genus *Leucochloridium*. The resemblance consists in the following features: the oval shape of the body, an intense development of the suckers, a similar course of the uterus, which has two loops on both sides of the ventral sucker*, a similar structure of the vitellaria made up of numerous very fine follicles.

But there are certain basic differences, too.

In the diagnoses of the subfamily *Leucochloridiinae* Poche, 1907 given by Sinitzin (1931) and Witenberg (1925) there are two main features: genital pore lying in the hind extremity of the body or on its dorsal side, vitellaria reaching the level of the bifurcation of the intestine or even in front of it.

In the fluke described by Sołtys and the metacercaria we found the genital pore lies ventrally, while the vitellaria reach only the level of the middle of the ventral sucker.

One should also stress certain biological differences, never taken account of in diagnoses before, between the species of the genus *Leucochloridium* known so far and the fluke described by Sołtys.

All flukes which unquestionably belong to the genus *Leucochloridium* parasitize in birds. Their development cycles which have been determined are very specific. Metacercariae develop inside a sporocyst which is usually characteristically coloured, and some authors consider the colour of the sporocyst to be a specific feature (Ginecinskaja, 1954). Nowhere in the studies on the development of various species of *Leucochloridium* have we found any references to these metacercariae occurring outside sporocysts. The

* Taking into account among other things the course of the uterus, Kagan (1951) distinguishes two genera: *Leucochloridium* Carus and *Neoleucochloridium* Kagan, 1951. Specimens in which the uterus transverses the body behind the ventral sucker would belong to the genus *Leucochloridium*, while those in which it transverses the body between the oral sucker and acetabulum would belong to the genus *Neoleucochloridium*.

number of metacercariae in a sporocyst is usually very high (some 100 to 300).

The metacercariae we found occurred in the kidney of snails without sporocysts and there were few of them (usually 1—2). A mature fluke parasitizes in mammals (*Sorex araneus*).

In 1953 Shaldibin described a new fluke species from the intestine of *Sorex araneus* and *Neomys fodiens* and called it *Leucochloridium skrjabini* Shaldibin. That species closely resembles the fluke described by Soltyś under the name of *Leucochloridium soricis* in the shape of the body, the size of the sucker, the range of the vitellaria, which never reach before the front border of the ventral sucker, and in the course of the uterus. Some of its organs have smaller dimensions (see Table VI), yet Soltyś gives no range of variability of various features in various specimens in his description. There is a distinct difference in the size of eggs, measuring 0.033×0.016 mm in *Leucochloridium soricis* and 0.027×0.015 in *L. skrjabini*. It follows from my measurements of 20 eggs of the same specimen of *L. soricis* that their size may vary: 0.026 — 0.0336×0.011 — 0.016 mm. Another difference consists in the position of the genital pore, which according to Shaldibin's description lies

Table VI

Pseudoleucochloridium soricis (Soltyś) and *Leucochloridium skrjabini* Shaldibin (in mm)

	<i>Pseudoleucochloridium soricis</i>		<i>Leucochloridium skrjabini</i>
	metacercaria	adult fluke	
length	0.72 — 1.6	1.7	0.952 — 1.530
width	0.52 — 0.96	0.9	0.475 — 0.635
oral sucker	0.21—0.32 × 0.22—0.43	0.40	0.204 — 0.340
ventral sucker	0.24—0.46 × 0.25—0.49	0.38	0.221 — 0.476
pharynx	0.07—0.12 × 0.10—0.16	0.12	0.102 — 0.153
testis I	0.06—0.18 × 0.08—0.149	0.16	0.102×0.102 — 0.119×0.136
testis II	0.06—0.13 × 0.06—0.152	—	0.153×0.153 — 0.153×0.170
ovary	0.05—0.16 × 0.07—0.13	0.12	0.085×0.085 — 0.15×0.136
eggs	—	0.033×0.016	0.027×0.015
position of genital pore	ventral	ventral	dorsal

dorsally in *L. skrjabini*. It appears that it would be good to check the position of this pore. Should it prove that it lies ventrally, the species should be assigned to the synonyms of *Pseudoleucochloridium soricis*.

Some analogy may also be established between the species discussed and representative of the genus *Panopistus* Sinitzin, 1931. The genital pore also lies behind the posterior testis and ventrally in flukes of that genus. The vitellaria reach the level of the ventral sucker, but consist of a small number of large follicles. The uterus does not pass behind the ventral sucker. The suckers are not so strongly developed and both lie in the fore part of the body. The shape of the body is more like that of representatives of the genus *Brachylaemus*.

The development, established in detail for *Panopistus pricei* Sinitzin by Krull (1935a), Reynolds (1938b) and Villella (1954), takes place in two intermediate hosts. Metacercariae develop in the kidney of the snail outside a sporocyst, just as in the fluke described by Sołtys under the name of *Leucochloridium soricis*. Adult flukes parasitize in mammals (*Blarina brevicauda*) as well.

It follows from our analysis that the fluke discussed is morphologically closer to the genus *Leucochloridium* and biologically closer to the genus *Panopistus*. However it differs from representatives of both of these genera in a few basic features and consequently we think it proper to establish it into a new genus, which we propose to call *Pseudoleucochloridium* nov. gen. in view of its great morphological resemblance to the genus *Leucochloridium*.

The genus *Pseudoleucochloridium* gen. nov.

Diagnosis: *Brachylaemidae*, unarmed, with an oval body and strongly developed suckers. Ventral sucker lying more or less in the middle of the body. Genital pore lying behind the posterior testis, ventrally. The uterus reaches the bifurcation of the intestine and surrounds the ventral sucker with two lateral loops. Vitellaria, composed of numerous small follicles, do not reach behind the anterior border of the ventral sucker.

Parasites of mammals. Metacercaria develops in the snail's kidney without a sporocyst.

Typical species of the genus: *Pseudoleucochloridium* gen. nov. *soricis* (Sołtys, 1951) occurring in the area of the Białowieża National Park.

It is rather difficult to assign this genus to respective subfamily. Although there is a great morphological similarity to representatives of the subfamily *Leucochloridiinae*, there are biological reasons against the assignment of this genus to this subfamily*. We leave this question open until the full development cycle of *Pseudoleucochloridium soricis* has been observed and more data on the biology of this parasite obtained.

Metacercaria of *Leucochloridium*

Over 80 specimens of metacercaria were found in only one specimen of *Ganiodiscus ruderatus* (Müll.) out of 153 examined.

Description. A very small, oval metacercaria, covered with very fine spines all over and enclosed in a very delicate, transparent cyst (Fig. 5b). Suckers very large, the oral usually larger than the

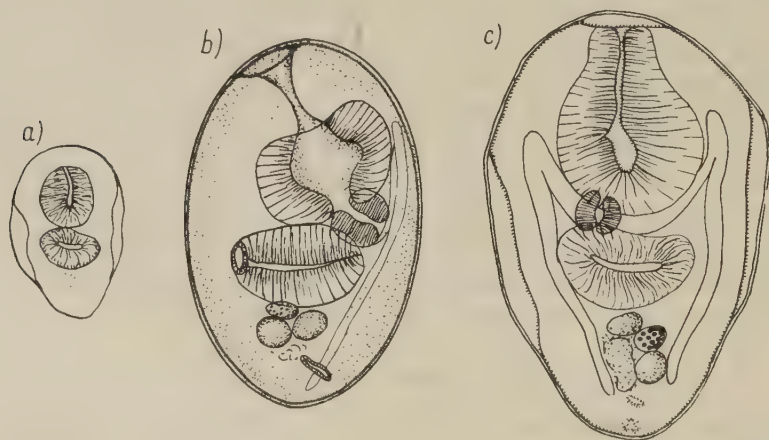


Fig. 5. Metacercaria of *Leucochloridium* sp.; a — The same magnifying as previous figures; b — Bigger magnifying; c — Ventral arrangement of pores of suckers and dorsal arrangement of genital pore — lateral view.

* There are similar difficulties in the case of *Panopistus* Sinitzin. Mehra (1936) transferred this genus to the subfamily *Leucochloridiinae* Poche, 1907. Ulmer, however, is right in pointing out that the morphology of larval stages and the parasitizing in mammals by adult from suggest closer relationship with the subfamily *Brachylaeminae*. Closer examination of larval stages of *Pseudoleucochloridium soricis* (Sołtys) may make it possible to set off these two genera as a common subfamily.

ventral. The opening of oral sucker lying terminally, the sucker itself usually deeply retracted. Pharynx lying immediately behind oral sucker (and in the preparations usually partly covering it and the ventral sucker), much smaller than the suckers (for dimensions see Table VII). Ventral sucker lying somewhat behind the middle of the body, its opening extended into a transverse slit. Intestinal crura narrow, first going to the sides and upwards, then forming a loop and going down backwards along the sides of the body without reaching its end. Reproductive system lying between intestinal crura and ventral sucker. Testes and ovary of almost the same size, spherical, arran-

Table VII
Dimensions of a live and a preserved metacercaria

Measurements	live specimens	preserved specimens
length	0.52	0.26 — 0.35
width	0.40	0.156 — 0.209
oral sucker	0.162×0.162	$0.068 — 0.099 \times 0.099 — 0.122$
ventral sucker	0.154×0.121	$0.066 — 0.079 \times 0.082 — 0.138$
pharynx	0.077×0.04	$0.026 — 0.041 \times 0.041 — 0.056$
testes	—	0.016 — 0.033
ovary	—	0.021 — 0.033

ged in a triangle. The course of the uterus and the vittellaria was not observed. Genital pore lying on the dorsal side, behind the posterior testis, on the level of, or somewhat behind, the ends of the intestine. Excretory pore lying terminally or also shifted to the dorsal side.

Fig. 5a shows the outlines of the metacercaria magnified just as those of the metacercariae of other species (Figs. 1—5). Fig. 5b shows the same fluke more magnified. The fluke shown in Fig. 5c lies somewhat sideways, owing to which the ventral position of the openings of the suckers and the dorsal position of the genital pore are very distinct.

Discussion. The general appearance of the body, the armature of the cuticle, the strong development of the suckers, and the dorsal position of the genital pore suggest that the described metacercaria belong to the genus *Leucochloridium*. The great number of metacercariae found in the snail would also suggest the same. Unfortunately, while crushing the snail we failed to observe in what organ the metacercariae occurred.

The poor development of the genital system did not allow an analogy to be drawn with the other *Leucochloridium* species described. Very small dimensions of this metacercaria were the only characteristic feature. The *Leucochloridium paradoxum* metacercaria found by us (Pojmańska, 1958) were twice as large. We cannot determine the species of this metacercaria on the basis of these poor data.

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STRESZCZENIE

1. Autorka przebadła 18 gatunków ślimaków lądowych z Białowieskiego Parku Narodowego; tylko u czterech znalazła metacerkarie (tabl. I). Intensywność i ekstensywność zarażenia były małe.

Znalezione metacerkarie były badane przyżyciowo przy użyciu czerwieni obojętnej i po utrwaleniu na preparatach. Przywry zabijano przez podgrzanie w kropli wody.

3. U ślimaka *Zonitoides nitidus* (Müll.) stwierdzono występowanie metacerkarii *Brachylaemus fulvus* Duj., 1843 (rys. 1, tab. II). Autorka uważa za synonimy tego gatunku: *Brachylaimus migrans* var. *a* Duj., 1845, *Panopistus europaeus* Sołtys, 1951, *Brachylaemus oesophagei* Szaldibin, 1953, oraz prawdopodobnie *Harmostomum dujardini* Baer, 1928.

4. U ślimaków *Succinea putris* (L.) i *Perforatella bidens* (Chemn.) znaleziono metacerkarię *Brachylaemus spinosulus* Hofmann, 1899 (rys. 2—4, tab. III). Eksperymentalnie uzyskano dorosłą przywrę z tych metacerkarii w jeżu (*Erinaceus europaeus* L.). Autorka zastanawia się na celowością wyróżniania dwóch gatunków: *Brachylaemus spinosulus* Hofmann, 1899 i *B. virginiana* (Dickerson) Krull, 1934 (tab. IV).

5. W tych samych gatunkach ślimaków stwierdzono występowanie metacerkarii przywry opisanej przez Sołtysa pod nazwą *Leucochloridium soricis* Sołtys, 1951 (rys. 4, tab. V). Na podstawie położenia otworu płciowego na stronie brzusznej, zasięgu żółtników do poziomu przyssawki brzusznej, oraz danych z biologii i rozwoju tego pasożyta, autorka wydzieliła ten gatunek w nowy rodzaj *Pseudoleucochloridium* nov. gen. Autorka przypuszcza, że opisany przez Szaldibina gatunek *Leucochloridium skrabini* jest identyczny z *P. soricis* (tab. VI).

6. W ślimaku *Goniodiscus ruderatus* (Müll.) znaleziono metacerkarie *Leucochloridium* sp. (rys. 5, tab. VIII). Przynależności gatunkowej tej przywry nie udało się określić.

